

### **AMENDMENT TO THE SPECIFICATION**

Please amend the title as the following:

#### **CRYSTALLIZATION OF MVAS (HMG-COA SYNTHASE)**

Please amend the paragraphs [0054], [0090], [00178], [00179], and [00183], Tables 2-4 on page 14-15 in the specification as the followings:

[0054] For example, based on the crystal structure, applicants have determined that the MvaS amino acids shown in Table 2 encompass a 4-Angstrom radius around the MvaS (SEQ ID NO:1) active site and thus likely to interact with any active site inhibitor of MvaS. Applicants have also determined that the amino acids of Table 3 encompass a 7-Angstrom radius around the MvaS (SEQ ID NO:1) active site. Further it has been determined that the amino acids of Table 4 encompass a 10-Angstrom radius around the MvaS active site. It is noted that there is one MvaS molecule in the asymmetric unit, referred to as chain A. Structural coordinates appear in Figure 3. It is noted that the sequence and structure of the residues in the active site may also be conserved and hence pertinent to other MvaS and homologs.

**Table 2:** Amino Acids encompassed by a 4-Angstrom radius around the MvaS (SEQ ID NO:1) active site.

ASP 29	GLY 31	LYS 32
GLY 36	GLU 79	ALA 110
CYS 111	TYR 143	GLY 148
GLY 149	THR 152	PHE 185
VAL 196	GLY 198	SER 201
ASN 202	TYR 205	HIS 233
PRO 235	TYR 236	MSE 239
LYS 242	ASN 275	TYR 306
GLY 307	SER 308	

**Table 3:** Amino Acids encompassed by a 7-Angstrom radius around the MvaS (SEQ ID NO:1) active site.

VAL 28	ASP 29	PRO 30
GLY 31	LYS 32	PHE 33
HIS 34	ILE 35	GLY 36
ILE 37	GLU 79	GLU 109
ALA 110	CYS 111	TYR 112
ASP 139	TYR 143	SER 147
GLY 148	GLY 149	GLU 150
PRO 151	THR 152	ASP 184
PHE 185	TRP 186	PRO 194
VAL 196	ASP 197	GLY 198
PRO 199	LEU 200	SER 201
ASN 202	GLU 203	THR 204
TYR 205	ILE 206	HIS 233
ILE 234	PRO 235	TYR 236
LYS 238	MSE 239	LYS 242
ASN 275	TYR 277	THR 278
SER 280	TYR 306	GLY 307
SER 308	GLY 309	

**Table 4:** Amino Acids encompassed by a 10-Angstrom radius around the MvaS (SEQ ID NO:1) active site.

MSE 19	ALA 23	ASN 27
VAL 28	ASP 29	PRO 30
GLY 31	LYS 32	PHE 33
HIS 34	ILE 35	GLY 36
ILE 37	GLY 38	GLN 39
MSE 42	THR 78	GLU 79
SER 80	LYS 108	GLU 109
ALA 110	CYS 111	TYR 112
GLY 113	ALA 114	ASP 139
ILE 140	ALA 141	LYS 142
TYR 143	GLY 144	ASN 146
SER 147	GLY 148	GLY 149

GLU 150	PRO 151	THR 152
GLN 153	GLY 154	ILE 182
TYR 183	ASP 184	PHE 185
TRP 186	ARG 187	PRO 194
MSE 195	VAL 196	ASP 197
GLY 198	PRO 199	LEU 200
SER 201	ASN 202	GLU 203
THR 204	TYR 205	ILE 206
GLN 207	SER 208	PHE 232
HIS 233	ILE 234	PRO 235
TYR 236	THR 237	LYS 238
MSE 239	GLY 240	LYS 241
LYS 242	ALA 243	LEU 245
TYR 263	GLY 274	ASN 275
LEU 276	TYR 277	THR 278
GLY 279	SER 280	LEU 281
PHE 304	SER 305	TYR 306
GLY 307	SER 308	GLY 309
ALA 310	VAL 311	ALA 312

[0090] By performing submicroliter volume sized crystallization experiments, as detailed in U.S. Patent No. 6,296,673, effective crystallization conditions for forming crystals of a MvaS complex were obtained. In order to accomplish this, systematic broad screen crystallization trials were performed on an MvaS complex using the sitting drop technique. In each experiment, a 100nL mixture of MvaS complex and precipitant was placed on a platform positioned over a well containing ~~100nL~~ 100μL of the precipitating solution. Precipitate and crystal formation was detected in the sitting drops. Fine screening was then carried out for those crystallization conditions that appeared to produce precipitate and/or crystal in the drops.

[00178] The gene encoding residues 1-383 (from ~~SEQ ID No. 1~~ SEQ ID NO:1), which corresponds to the full-length MvaS from *E. faecalis*, was isolated by PCR from *E. faecalis* genomic DNA (ATCC700800D ) and cloned into the TOPO-activated cloning site of pSX26 vector. This DNA sequence is presented in ~~SEQ ID No. 2~~ SEQ ID NO:2. Expression in this vector generated a fusion of the full-length MvaS with non-cleavable carboxy-terminal six

histidine tag, the amino acid sequence of which is shown, underlined, in Figure 1 (~~SEQ. ID No. 1~~ SEQ ID NO:1). For production of seleno methionine labeled protein, the expression plasmid encoding for MvaS\_Ef fused with carboxy-terminal histidine tag was transformed into methionine auxotroph DL41 (Hendrickson, W. 1990. EMBO J. 9:1655-0).

[00179] Biomass for purification of recombinant seleno-methionine labeled MvaS\_Ef was generated using minimal media supplemented with seleno-methionine (Sigma, MO) using 96-well fermentor. It should be noted that a variety of other protocols and expression strains are also suitable for the expression of selenomethionine derivative of MvaS, website: cbr.med.harvard.edu/investigators/springer/lab/protocols/sara\_SeMet.html (~~http://cbr.med.harvard.edu/investigators/springer/lab/protocols/sara\_SeMet.html~~; Doublié, S. (1997)[[.]], *Methods in Enzymology* 276, 523-530; website: novagen.com/docs/ndis/INNO10-005.pdf ~~http://www.novagen.com/docs/ndis/INNO10-005.pdf~~), as would be readily appreciated by one of skill in the art. Cells from a single 70 ml fermentor tubes was thawed by addition of 21 ml of lysis buffer (50 mM Tris/HCl pH 7.9, 50 mM NaCl, 1 mM MgCl<sub>2</sub>) containing hen egg white lysozyme (0.6 mg/ml) and Benzonase (2.5 U/ml) and sonicated using Sonic Hedgehog robot. The sonicate was allowed to stand for 30 minutes at ~4°C. Total lysate was clarified by centrifugation and 2mL of 5M NaCl were added to the cleared lysate. The cleared lysate from four fermentor tubes was applied to 3 ml bed ProBond column that had been equilibrated to 50 mM Potassium Phosphate pH 7.8, 0.4 M NaCl, 0.1 M KCl, 20 mM imidazole, 10% glycerol, 0.25 mM TCEP. The solution was passed through the column using gravity flow and the column was washed with 6 bed volumes of 50 mM Potassium Phosphate pH 7.8, 0.4 M NaCl, 0.1 M KCl, 40 mM imidazole, 10% glycerol, 0.25 mM TCEP (Tris(2-carboxyethyl)phosphine hydrochloride). The product was eluted with 12 ml of 50 mM Potassium Phosphate pH 7.4, 0.4 M NaCl, 0.1 M KCl, 200 mM imidazole, 10% glycerol, 0.25mM TCEP. The eluted protein was concentrated and buffer-exchanged into 25 mM Tris pH 7.9, 150 mM NaCl by using Vivaspinn centrifugal concentrators. Following three five-fold dilution buffer-exchanges, the IMAC (immobilized metal affinity chromatography, Clontech, Mountain View, CA) purified MvaS\_Ef

Serial No.: 10/758,291  
Filing Date: 01/14/2004

was concentrated to 10 mg/ml with a total volume of 0.47 ml. The molecular weight of the purified protein corresponded to the 100% incorporation of seleno-methionine as determined by Mass Spectrograph (MS) analysis (43,443 observed and 43,436 expected without N-terminal methionine). Purified MvaS\_Ef exhibited a major band by both isoelectric focusing (IEF) and by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analyses.

[00183] Provided are crystals relating to MvaS (HMG-CoA Synthase) and its various uses.